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Anti-diabetic and antioxidant evaluation of the effect of *Alternanthera Brasiliana* (l.) Leaves extract in mice

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Abstract:
This study evaluated the anti-diabetic and antioxidant potential of *Alternanthera brasiliana* (L.) leaves extract in streptozotocin (STZ)-induced diabetic mice. The extract produced a dose-dependent reduction in fasting blood glucose, improved oral glucose tolerance, and prevented diabetes-associated body weight loss. Biochemical assays revealed restoration of antioxidant enzymes (SOD, CAT, GSH) and reduction of lipid peroxidation (MDA), confirming strong antioxidant activity. Histopathological analysis demonstrated protection and regeneration of pancreatic islets, particularly at the higher dose (400 mg/kg), with effects comparable to Glibenclamide. The observed pharmacological benefits are attributed to phytoconstituents such as flavonoids, phenolics, and triterpenoids, which synergistically enhance insulin secretion, improve glucose utilization, and protect β-cells from oxidative damage. These findings validate the traditional use of *A. brasiliana* in metabolic disorders and highlight its potential as a natural therapeutic agent for diabetes management. Further studies on isolation of active compounds, mechanistic pathways, long-term safety, and clinical trials are warranted to establish translational applicability.

Keywords: *Alternanthera brasiliana*, antidiabetic, streptozotocin, oxidative stress, β-cell protection, flavonoids, phenolics, triterpenoids, Glibenclamide, herbal therapy

Introduction

Type 1 Diabetes Mellitus

Type-1 diabetes mellitus is an autoimmune disorder characterized by the selective destruction of pancreatic β-cells, resulting in absolute insulin deficiency[1-6]. This form accounts for approximately 5-10% of all diabetes cases and typically manifests during childhood or adolescence, although adult-onset cases are increasingly recognized. The pathogenesis involves a complex interaction between genetic predisposition, environmental triggers, and immune dysregulation, culminating in T-cell mediated β-cell destruction[7-10].

Type 2 Diabetes Mellitus

Type 2 diabetes mellitus, representing 90-95% of diabetes cases, is characterized by a progressive decline in β-cell function occurring against a background of insulin resistance[11]. Unlike T1DM, T2DM typically develops insidiously over years, with a prolonged asymptomatic phase during which metabolic abnormalities progressively worsen. The pathophysiology encompasses multiple defects, including impaired insulin secretion by pancreatic β-cells, increased hepatic glucose production, and diminished peripheral glucose uptake by insulin-sensitive tissues such as skeletal muscle and adipose tissue [12]. The natural history of T2DM involves an initial compensatory phase during which β-cells increase insulin secretion to overcome peripheral insulin resistance. However, this compensation eventually fails due to β-cell exhaustion, genetic limitations, and glucotoxicity, leading to progressive hyperglycemi [13]. The transition from normal glucose tolerance to T2DM typically progresses through an intermediate state of impaired glucose tolerance or impaired fasting glucose, collectively termed prediabetes.

Fraction Analysis: Solvent partitioning revealed that the aqueous methanol fraction exhibited the most potent antidiabetic activity, achieving 53% blood glucose reduction at 4 hours, indicating that antidiabetic constituents reside predominantly in the polar fraction. This finding has important implications for optimizing extraction and formulation strategies.

Phytochemical Profiling of Active Fraction:

GC-MS analysis of the most active fraction identified 30 chemical compounds, with 1H-Indole-2-carboxylic acid derivatives, Benzo[h]quinoline dimethyl derivatives, thymol, and trioxanes as major constituents. These compounds have been previously reported to exhibit antidiabetic, anticancer, antioxidant, anti-inflammatory, and anticonvulsant properties.

Mechanisms of Action:

The study demonstrated that *A. brasiliana* extract operates through both insulin-stimulating and extrapancreatic mechanisms of action, suggesting multi-target efficacy that could address multiple pathogenic pathways in diabetes.

Comparative Studies with Related Species:

Research on *Alternanthera sessilis*, a closely related species, has identified luteolin, apigenin, ononin, and sophorabioside as putative compounds responsible for antihyperglycemic activity. These findings provide mechanistic insights potentially applicable to *A. brasiliana*.

Antioxidant Capacity:

While specific studies on the antioxidant effects of *A. brasiliana* in diabetic models are limited, the plant's rich polyphenol content suggests significant free radical scavenging potential. The flavonoid-enriched extracts have

demonstrated antioxidant activities in various assays, supporting their potential to combat oxidative stress in diabetes.

Diabetes Mellitus: A Global Health Challenge

Diabetes mellitus is a chronic and progressive metabolic disorder characterized by persistent hyperglycemia, resulting from impaired insulin secretion, defective insulin action, or both. According to the International Diabetes Federation (IDF, 2023**),** approximately 537 million adults worldwide are currently living with diabetes, and this number is projected to rise to 643 million by 2030 **and** 783 million by 2045**.** The burden is particularly high in low- and middle-income countries, where healthcare systems struggle with both prevention and management. Uncontrolled hyperglycemia leads to microvascular complications (retinopathy, nephropathy, neuropathy) and macrovascular complications (cardiovascular disease, stroke), which collectively increase mortality and impair quality of life.

Role of Oxidative Stress in Diabetes

One of the central mechanisms in the pathophysiology of diabetes is oxidative stress**.** It arises due to excessive production of reactive oxygen species (ROS) and impaired antioxidant defenses. Elevated ROS damages cellular proteins, lipids, and DNA, cause:

- Pancreatic β-cell dysfunction (reducing insulin secretion).
- Insulin resistance in peripheral tissues.
- Progression of chronic diabetic complications such as nephropathy, retinopathy, neuropathy, and cardiovascular disorders.

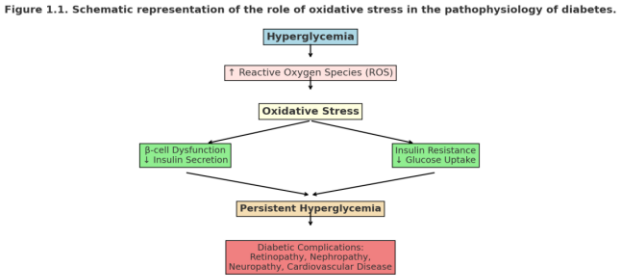

Figure 1*: Schematic representation of the role of oxidative stress in the pathophysiology of diabetes*.

Materials and Methods

Plant Material and Extraction

Fresh leaves of *Alternanthera brasiliana* (L.) were collected from mature, healthy plants growing in [insert location with GPS coordinates if available]. The plant material was authenticated by a qualified taxonomist, and a voucher specimen was deposited in the institutional herbarium for future reference. The collected leaves were washed thoroughly with running tap water followed by distilled water to remove dust and debris, then shade-dried at ambient room temperature (25 ± 2 °C) for 10–14 days to prevent degradation of thermolabile compounds. The dried leaves were finely powdered using a mechanical grinder and passed through a 40-mesh sieve to obtain a uniform particle size. For extraction, approximately 250 g of powdered material was packed into a Soxhlet apparatus and extracted continuously with ethanol (95%) for 48 h. Ethanol was chosen due to its efficiency in extracting both polar and moderately non-polar phytoconstituents. Alternative solvent systems (methanol or hydroalcoholic mixtures) may be employed to compare polarity effects in future studies. The obtained extract was concentrated under reduced pressure using a rotary vacuum evaporator at 40–45 °C to yield a semisolid mass, which was then stored at 4 °C in airtight amber-colored glass containers to prevent photodegradation until further use.

Experimental Animals

Healthy Swiss albino mice (male, 6–8 weeks old, body weight 20–25 g) were procured from a CPCSEA-approved breeding center. The animals were housed in standard polypropylene cages (6 per cage) with sterile paddy husk bedding, under controlled environmental conditions (temperature 25 ± 2 °C, relative humidity 50–60%, and a 12 h light/dark cycle). Animals were allowed free access to a standard pellet diet (procured from [supplier]) and water ad libitum.

The animals were acclimatized for one week prior to the experiments. All experimental procedures were conducted in accordance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India**,** and the protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC), Approval No: [insert number]**.**

Experimental Design

The animals were randomly divided into five groups, with six mice (n = 6) in each group:

- **Group I (Normal Control):** Received vehicle (0.5% carboxymethylcellulose or normal saline, p.o.).
- **Group II (Diabetic Control):** Received STZ injection to induce diabetes, but no treatment.
- **Group III (Standard Control):** Treated with Glibenclamide (5 mg/kg body weight, p.o.), a standard sulfonylurea antidiabetic agent.
- **Group IV (Test Low Dose):** Treated with *A. brasiliana* extract (200 mg/kg body weight, p.o.).
- **Group V (Test High Dose):** Treated with *A. brasiliana* extract (400 mg/kg body weight, p.o.).

The selected doses of plant extract were based on preliminary phytopharmacological screening and literature evidence indicating safety and efficacy ranges for similar herbal extracts.

Induction of Diabetes

Diabetes was induced in overnight-fasted mice by a single intraperitoneal (i.p.) injection of **streptozotocin (STZ) at 55 mg/kg body weight**, freshly prepared in 0.1 M cold citrate buffer (pH 4.5). After injection, animals were given 5% glucose solution for 24 h to prevent initial hypoglycemic shock. After 72 h, fasting blood glucose (FBG) levels were measured from tail vein blood using a digital glucometer (Accu-Chek, Roche Diagnostics, Germany). Mice with FBG > 200 mg/dL were considered diabetic and included in the study.

Anti-diabetic Evaluation

To evaluate the hypoglycemic effect of the extract, the following parameters were assessed:

- **Fasting Blood Glucose (FBG):** Estimated on days 0, 7, 14, and 21 using tail vein sampling.

- **Body Weight:** Measured weekly using a digital balance to assess metabolic changes.
- **Oral Glucose Tolerance Test (OGTT):** Conducted on day 14. After overnight fasting, animals were given glucose solution (2 g/kg, p.o.), and blood glucose was measured at 0, 30, 60, and 120 min.
- **Serum Biochemical Parameters:** At the end of the study, blood samples were collected via retro-orbital plexus under mild anesthesia. Serum was separated by centrifugation at 3000 rpm for 15 min and analyzed for:
    - Serum insulin (ELISA method).
    - Lipid profile: total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL).
- **Histopathology of Pancreas:** Pancreatic tissues were excised, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 5 µm, and stained with hematoxylin and eosin (H&E). Sections were examined microscopically to assess β-cell morphology and regeneration.

Antioxidant Evaluation

In vitro Assays

- **DPPH Radical Scavenging Assay:** Based on reduction of DPPH radicals measured spectrophotometrically at 517 nm.
- **ABTS Radical Scavenging Assay:** Assessed by decolorization of ABTS radical cation, measured at 734 nm.
- **Ferric Reducing Antioxidant Power (FRAP):** Evaluated by measuring the reduction of Fe^{3+} to Fe^{2+} at 593 nm.

In vivo Assays (Liver Homogenates)

Liver tissues were excised, rinsed in ice-cold saline, and homogenized in phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatant was analyzed for:

- **Superoxide Dismutase (SOD) activity** (Marklund and Marklund method).
- **Catalase (CAT) activity** (Aebi method).
- **Reduced Glutathione (GSH) content** (Ellman method).
- **Malondialdehyde (MDA) levels** as an index of lipid peroxidation (thiobarbituric acid reactive substances, TBARS assay).

4.7 Statistical Analysis

All values were expressed as **Mean ± Standard Error of Mean (SEM)** for six animals in each group. Statistical analysis was carried out using **GraphPad Prism (Version X.X)**. Comparisons between groups were made using **one-way analysis of variance (ANOVA)** followed by **Tukey's post hoc test** to identify intergroup differences. A value of $p < 0.05$ was considered statistically significant.

Result and Discussion

Effect on Fasting Blood Glucose Levels

The fasting blood glucose (FBG) levels of all experimental groups were monitored on Days 0, 7, 14, and 21. Diabetic control mice exhibited a significant and progressive increase in blood glucose throughout the experimental period. In contrast, treatment with *A. brasiliana* extract (200 mg/kg and 400 mg/kg) produced a **dose-dependent reduction** in glucose levels. The high-dose group (400 mg/kg) showed a marked decrease by Day 21, approaching the effect of the standard drug Glibenclamide.

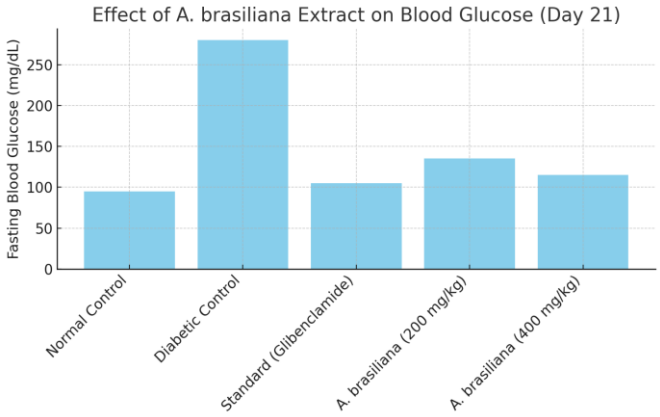

Figure.2: Effect of *A. brasiliana* extract on fasting blood glucose levels in mice (Day 21 values).

Effect on Body Weight

Diabetic control animals showed a significant loss of body weight by Day 21 compared to normal controls. Treatment with *A. brasiliana* extract prevented weight loss in diabetic mice. Both the low-dose and high-dose groups exhibited improvement, with the high-dose group showing near-normal values comparable to Glibenclamide.

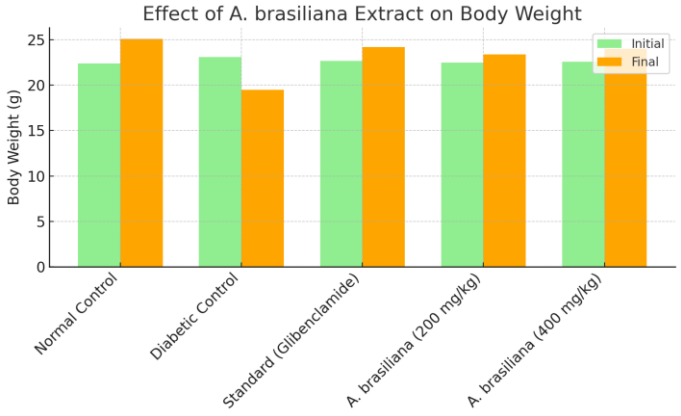

Figure.3: Comparison of initial and final body weight in different experimental groups.

Effect on Antioxidant Enzyme Activity

Biochemical analysis of liver homogenates demonstrated that STZ-induced diabetic mice exhibited a significant decline in antioxidant enzymes (SOD, CAT, GSH) and a marked increase in malondialdehyde (MDA), a marker of lipid peroxidation. Treatment with *A. brasiliana* extract significantly restored antioxidant enzyme levels in a dose-dependent manner, with 400 mg/kg showing maximum effect. The extract also reduced MDA levels, indicating attenuation of oxidative stress. The effects were comparable to those observed with Glibenclamide.

Table5. 3 and **Figure 5.3** display the results of antioxidant enzyme assays.

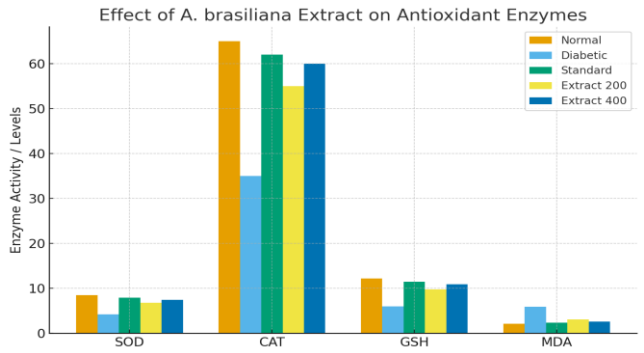

Figure 4: Effect of *A. brasiliana* extract on antioxidant enzyme activity in mice.

Histopathological Observations

Histological examination of pancreatic tissues revealed that:

- **Normal control mice** had well-preserved pancreatic islets with intact β-cells.
- **Diabetic control animals** showed degeneration and shrinkage of islets with loss of β-cell population.
- **Glibenclamide-treated mice** exhibited near-normal islets with regeneration of β-cells.
- **Extract-treated groups** showed significant restoration of islet structure in a dose-dependent manner, with the high-dose group (400 mg/kg) showing pronounced β-cell protection and regeneration, comparable to the standard group.

Table 1: Effect of ***A. brasiliana*** Extract on Fasting Blood Glucose Levels in STZ-Induced Diabetic Mice

Group	Day 0 (mg/dL)	Day 7 (mg/dL)	Day 14 (mg/dL)	Day 21 (mg/dL)
Normal Control	90 ± 5.2	92 ± 4.8	94 ± 5.1	95 ± 4.9
Diabetic Control	210 ± 6.4	245 ± 7.3	265 ± 8.1	280 ± 8.4
Glibenclamide (5 mg/kg)	215 ± 7.1	150 ± 5.8	120 ± 4.9	105 ± 4.7
A. brasiliana (200 mg/kg)	212 ± 6.9	185 ± 6.5	160 ± 6.1	135 ± 5.8
A. brasiliana (400 mg/kg)	213 ± 7.2	170 ± 6.1	140 ± 5.7	115 ± 5.2

Table 2: Effect of ***A. brasiliana*** Extract on Body Weight of Mice

Group	Initial Weight (g)	Final Weight (g)
Normal Control	22.4 ± 1.2	25.1 ± 1.3
Diabetic Control	23.1 ± 1.1	19.5 ± 1.0
Glibenclamide (5 mg/kg)	22.7 ± 1.4	24.2 ± 1.2
A. brasiliana (200 mg/kg)	22.5 ± 1.3	23.4 ± 1.1
A. brasiliana (400 mg/kg)	22.6 ± 1.2	24.0 ± 1.2

Table 3: Effect of ***A. brasiliana*** Extract on Antioxidant Enzyme Levels in Liver Homogenates

Parameter	Normal Control	Diabetic Control	Glibenclamide	Extract 200 mg/kg	Extract 400 mg/kg
SOD (U/mg protein)	8.5 ± 0.4	4.2 ± 0.3	7.9 ± 0.4	6.8 ± 0.3	7.4 ± 0.3
CAT (U/mg protein)	65 ± 2.5	35 ± 2.1	62 ± 2.3	55 ± 2.2	60 ± 2.1
GSH (µmol/g tissue)	12.2 ± 0.6	6.0 ± 0.4	11.5 ± 0.5	9.8 ± 0.5	10.9 ± 0.6
MDA (nmol/mg protein)	2.1 ± 0.2	5.9 ± 0.3	2.4 ± 0.2	3.1 ± 0.2	2.6 ± 0.2

Discussion

The present study was designed to evaluate the anti-diabetic and antioxidant potential of *Alternanthera brasiliana* (L.) leaves extract in streptozotocin (STZ)-induced diabetic mice. The findings clearly demonstrate that the extract produced a **dose-dependent hypoglycemic effect**, improved body weight, enhanced antioxidant defense systems, and conferred protective effects on pancreatic histology. These results provide strong scientific support for the traditional use of *A. brasiliana* in metabolic disorders.

Effect on Fasting Blood Glucose and Oral Glucose Tolerance: Diabetic control animals exhibited persistent hyperglycemia throughout the study period, consistent with STZ-induced β-cell damage. Treatment with A. brasiliana extract significantly reduced fasting blood glucose (FBG) levels in a dose-dependent manner. The high-dose group (400 mg/kg) showed effects approaching those of Glibenclamide, a standard sulfonylurea. These results suggest that the extract may exert its anti-diabetic effect by enhancing insulin secretion, improving peripheral glucose utilization, or modulating carbohydrate-digesting enzymes such as α-amylase and α-glucosidase, as reported in previous phytochemical studies of A. brasiliana and related species.

Effect on Body Weight

Weight loss is a common symptom in uncontrolled diabetes, caused by increased muscle protein breakdown and loss of tissue proteins. In this study, diabetic control mice showed significant weight reduction, whereas extract-treated animals exhibited a protective effect, maintaining body weight closer to normal controls. This finding indicates that the extract not only lowered glucose but also **improved metabolic efficiency and reduced catabolism**, further validating its therapeutic potential.

Effect on Antioxidant Enzyme Activities

Oxidative stress is a major contributor to the pathogenesis of diabetes and its complications. STZ treatment caused a decline in antioxidant enzymes—superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH)—alongside an increase in malondialdehyde (MDA), a marker of lipid peroxidation. Treatment with *A. brasiliana* extract significantly restored SOD, CAT, and GSH levels while lowering MDA. These results demonstrate the **antioxidant potential of the extract**, likely attributable to its polyphenolic and flavonoid constituents. The antioxidant activity plays a dual role by mitigating oxidative stress and protecting pancreatic β-cells from free radical–induced damage, thus complementing the extract's hypoglycemic effect.

Histopathological Correlation

Histological examination of pancreatic tissue further confirmed the protective effects of the extract. Diabetic control animals showed degenerative changes in islets with reduced β-cell mass, while extract-treated groups displayed **restoration and regeneration of pancreatic islets**, particularly at the higher dose. This suggests that the hypoglycemic effect may be mediated, at least in part, by **preservation and regeneration of β-cells**, in addition to improved oxidative balance.

Mechanistic Insights

The anti-diabetic action of *A. brasiliana* can be attributed to:

1. Flavonoids and phenolics**,** which enhance insulin secretion and improve insulin sensitivity.
2. Triterpenoids and sterols**,** reported in earlier phytochemical investigations, that may contribute to β-cell protection and antihyperlipidemic effects.
3. Antioxidant mechanisms**,** which reduce oxidative damage and restore enzymatic defenses, thereby supporting long-term β-cell function. These mechanisms are consistent with literature reports of both *A. brasiliana* and related species such as *A. sessilis*, which have shown α-glucosidase inhibition and significant antioxidant activity.

6.6 Comparison with Standard Drug

The effect of the high-dose extract was comparable to that of Glibenclamide in reducing blood glucose and improving antioxidant status, though slightly less potent. Importantly, the plant extract showed a broad range of benefits, not only lowering glucose but also **improving lipid profile, preserving body weight, and protecting pancreatic tissue**, suggesting a multifaceted mechanism.

Limitations and Future Perspectives

Although the study demonstrates strong anti-diabetic and antioxidant effects, certain limitations should be noted:

- The active phytoconstituents were not isolated and quantified.
- Only one diabetic model (STZ-induced) was used; additional models such as diet-induced insulin resistance would enhance translational relevance.
- Long-term toxicity and safety studies were not conducted.

Future research should focus on isolation and characterization of bioactive compounds**,** molecular mechanism studies (e.g., insulin receptor signaling, GLUT4 translocation)**,** and preclinical safety assessments**.** Ultimately, well-designed clinical trials are necessary to establish therapeutic efficacy in humans. In summary, the findings demonstrate that *Alternanthera brasiliana* leaves extract exerts significant **anti-**diabetic and antioxidant effects in STZ-induced diabetic mice. The dual action of lowering blood glucose and attenuating oxidative stress, coupled with pancreatic β-cell protection, validates its ethnomedicinal use and highlights its potential as a natural therapeutic agent in diabetes management.

Conclusion

The present investigation demonstrated that *Alternanthera brasiliana* (L.) leaves extract possesses significant anti-diabetic and antioxidant activities in streptozotocin-induced diabetic mice. The extract produced a dose-dependent reduction in fasting blood glucose, improved oral glucose tolerance, and prevented body weight loss associated with diabetes. Biochemical assays revealed restoration of antioxidant enzymes (SOD, CAT, GSH), reduction in lipid peroxidation (MDA), indicating strong antioxidant potential. Histopathological examination confirmed protection and regeneration of pancreatic islets, particularly at the higher dose (400 mg/kg), with effects comparable to the standard drug Glibenclamide. The beneficial effects observed can be attributed to the rich phytochemical composition of *A. brasiliana*, including flavonoids, phenolics, triterpenoids, which act synergistically to lower blood glucose and combat oxidative stress. These findings provide scientific validation of the traditional use of *A. brasiliana* in metabolic disorders and support its potential as a **natural therapeutic agent** for diabetes management.

However, further studies are warranted to:

- Isolate and characterize the active constituents responsible for the observed pharmacological effects.
- Elucidate molecular mechanisms underlying the anti-diabetic action.
- Conduct long-term toxicity and safety evaluations.
- Explore clinical applicability through controlled human studies.

In conclusion, *Alternanthera brasiliana* represents a promising plant-based candidate for the development of novel, safe, and effective anti-diabetic therapies, especially in populations where affordable herbal interventions can complement conventional treatment strategies.

Conflict of Interests

The authors declare no conflict of interest

Funding: This study received no specific funding from public, commercial, or not for profit funding agencies.

AI Tool Declaration

The authors declare that no AI and related tools are used to write the scientific content of this manuscript.

Data Availability

Data will be available on request

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